

(19)



Europäisches Patentamt  
European Patent Office  
Office européen des brevets



(11)

EP 0 585 988 B1

(12)

**EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention  
of the grant of the patent:  
13.03.1996 Bulletin 1996/11

(51) Int. Cl.<sup>6</sup>: **A21D 8/04**

(21) Application number: 93202197.5

(22) Date of filing: 23.07.1993

**(54) Enzyme product and method for improving bread quality**

Enzymprodukt und Verfahren zur Verbesserung der Qualität von Brot  
Produit enzymatique et méthode d'amélioration de la qualité du pain

(84) Designated Contracting States:  
AT BE CH DE DK ES FR GB GR IE IT LJ LU MC NL  
PT SE

• Docter, Cees,  
c/o Gist Brocades N.V.  
NL-2600 MA Delft (NL)

(30) Priority: 27.07.1992 EP 92202315

(74) Representative: Matulewicz, Emil Rudolf  
Antonius, Dr. et al  
Gist-Brocades NV  
Patents and Trademarks Department  
Wateringseweg 1  
P.O. Box 1  
NL-2600 MA Delft (NL)

(43) Date of publication of application:  
09.03.1994 Bulletin 1994/10

(73) Proprietor: GIST-BROCADES N.V.  
NL-2600 MA Delft (NL)

(72) Inventors:  
• Van Eljk, Jan Henricus  
NL-3722 AT Bilthoven (NL)

(56) References cited:  
EP-A- 0 468 731                      EP-A- 0 487 122  
US-A- 4 160 848

EP 0 585 988 B1

Note: Within nine months from the publication of the mention of the grant of the European patent, any person may give notice to the European Patent Office of opposition to the European patent granted. Notice of opposition shall be filed in a written reasoned statement. It shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).

therefore, that excellent bread quality is obtained when using this enzyme combination without an emulsifier.

Table 1

Addition	loaf volume (ml)	crumb firmness after 72 hours (g)
1. shortening (0%)	3500	900
2. shortening (0%) distilled mono- glycerides (0.5%)	3500	720
3. shortening (0%) lipase (2000 U/kg flour)	3200	950
4. shortening (0%) fungal amylase (90 FAU/kg flour) hemicellulase (105 U/kg flour)	3800	650
5. shortening (0%) fungal amylase (90 FAU/kg flour) hemicellulase (105 U/kg flour) lipase (2000 U/kg flour)	3300	950
6. shortening (3%)	3600	710
7. shortening (3%) distilled mono- glycerides (0.5%)	3600	550
8. shortening (3%) lipase (2000 U/kg flour)	3600	560
9. shortening (3%) fungal amylase (90 FAU/kg flour) hemicellulase (105 U/kg flour)	4050	450
10. shortening (3%) fungal amylase (90 FAU/kg flour) hemicellulase (105 U/kg flour) lipase (2000 U/kg flour)	4100	350

EXAMPLE 2

Baking tests were carried out as described in Example 1. A recipe containing 3% shortening was used throughout this baking test and crumb firmness of the breads was measured as described in Example 1.

Table 2 shows the result of the baking test using various emulsifiers and enzyme combinations. Superior bread quality is obtained when adding a combination of amylase, hemicellulase and lipase. This Example demonstrates that bread improvers containing this enzyme combination have better performance than bread improvers containing conventional emulsifiers like SSL, DATA-esters or monoglycerides.

Table 2

Addition	loaf volume (ml)	crumb firmness after 72 hours (g)
1. fungal amylase (30 FAU/kg flour) hemicellulase (35 U/kg flour) distilled mono- glycerides (0.15%)	3700	580
2. fungal amylase (90 FAU/kg flour) hemicellulase (105 U/kg flour) distilled mono- glycerides (0.45%)	3900	400
3. fungal amylase (30 FAU/kg flour) hemicellulase (35 U/kg flour) SSL (0.1%)	3850	520
4. fungal amylase (90 FAU/kg flour) hemicellulase (105 U/kg flour) SSL (0.3%)	3950	415
5. fungal amylase (30 FAU/kg flour) hemicellulase (35 U/kg flour) DATA-ester (0.1%)	3950	500
6. fungal amylase (90 FAU/kg flour) hemicellulase (105 U/kg flour) DATA-ester (0.3%)	4000	410
7. fungal amylase (30 FAU/kg flour) hemicellulase (35 U/kg flour) lipase (1000 U/kg flour)	3900	450
8. fungal amylase (90 FAU/kg flour) hemicellulase (105 U/kg flour) lipase (3000 U/kg flour)	4100	320

EXAMPLE 3

Breads were baked with variable amounts of shortening and enzyme combination comprising fungal amylase (90 FAU/kg flour), hemicellulase (105 U/kg flour) and lipase (3000 U/kg flour). The results (see Table 3) show that the amount of shortening in the recipe is critical for obtaining the desired bread quality. Best results are obtained when using high levels of shortening, whereas solid shortening gives better results than liquid oil.

Table 3

Addition	loaf volume (ml)	crumb firmness after 72 hours (g)
1. shortening (0%)	3250	900
2. shortening (1%)	3900	450
3. soy bean oil (1%)	3400	790
4. shortening (3%)	4100	350
5. shortening (5%)	4050	330

EXAMPLE 4

White pan bread was prepared according to the following recipe for a sponge and dough process:

Sponge	Flour	70 %
	Yeast	2 %
	Yeast food	0.3%
	Water	38.5%
Dough	Flour	30 %
	Water	16.5%
	Sugar	5 %
	Salt	2 %
	Milkpowder	4 %
	Shortening	5 %

The sponge was prepared by mixing the ingredients into a dough using a Kemper spiral mixer (380 rotations at speed 1 followed by 700 rotations at speed 2). After a sponge fermentation of 4 hours at 28°C the sponge was returned into the mixer bowl, all of the dough ingredients were added and mixed into a dough (280 rotations at speed 1 followed by 700 rotations at speed 2 in the Kemper spiral mixer).

The dough of 29°C temperature was divided immediately after mixing into pieces of 620 g which were proofed during 25 minutes at 31°C, moulded, panned, proofed for 65 minutes at 43°C and baked for 25 minutes at 190°C. Crumb firmness was measured after 3 days storage according to the procedure described in Example 1. The results (Table 4) show that superior bread quality is obtained in a sponge and dough process, when adding a combination of lipase,

hemicellulase and amylase at this dough site.

Table 4

Addition (at dough site)	loaf volume (ml)	crumb firmness after 72 hours (g)
1. fungal amylase (112.5 FAU/kg flour)	2875	530
2. fungal amylase (112.5 FAU/kg flour) distilled monoglycerides (0.3%)	2870	440
3. fungal amylase (112.5 FAU/kg flour) hemicellulase (112 U/kg flour)	2950	480
4. fungal amylase (112.5 FAU/kg flour) hemicellulase (112 U/kg flour) lipase (1200 U/kg flour)	3000	360

#### Claims

1. A bread improver composition which comprises at least one lipase, at least one hemicellulase and at least one amylase.
2. A composition according to claim 1 wherein the lipase is derived from a Rhizopus, Aspergillus, Candida, Penicillium or Mucor strain.
3. A composition according to claim 1 or 2 wherein the hemicellulase is derived from an Aspergillus or Trichoderma strain.
4. A composition according to any one of the preceding claims wherein the amylase is an  $\alpha$ -amylase and preferably derived from Aspergillus oryzae.
5. A dough which comprises a composition as claimed in any one of the preceding claims, flour, water and yeast.
6. A dough according to claim 5 which further comprises shortening, preferably comprises 100 g or less of shortening per kg of flour and more preferably comprises from 15 to 30 g shortening per kg of flour.
7. A dough according to any one of claims 5 or 6 which comprises from 25 to 1250 fungal amylase units (FAU) of  $\alpha$ -amylase per kilogram of flour and preferably comprises from 75-250 FAU of  $\alpha$ -amylase per kilogram of flour.
8. A dough according to any one of claims 5 to 7 which comprises from 25 to 500  $\beta$ -xylanase units of hemicellulase per kilogram of flour and preferably comprises from 35 to 250  $\beta$ -xylanase units of hemicellulase per kilogram of flour.
9. A dough according to any one of the preceding claims which comprises from 450 to 4000 units lipase per kilogram of flour and preferably comprises from 500 to 2500 units lipase per kilogram of flour.
10. A dough according to any one of the preceding claims which is substantially free of emulsifiers.
11. A process for producing bread which comprises forming a dough as claimed in any one of claims 5 to 10 and baking the dough.

#### Patentansprüche

1. Backhilfsmittel, welches mindestens eine Lipase, mindestens eine Hemicellulase und mindestens eine Amylase umfasst.
2. Mittel nach Anspruch 1, in welchem die Lipase von einem Rhizopus-, Aspergillus-, Candida-, Penicillium- oder Mucor-Stamm abgeleitet ist.

EP 0 585 988 B1

3. Mittel nach Anspruch 1 oder 2, in welchem die Hemicellulase von einem Aspergillus- oder Trichoderma-Stamm abgeleitet ist.
- 5 4. Mittel nach einem der vorhergehenden Ansprüche, in welchem die Amylase eine  $\alpha$ -Amylase und vorzugsweise von Aspergillus oryzae abgeleitet ist.
5. Teig, welcher Mehl, Wasser, Hefe und ein Mittel nach einem der vorhergehenden Ansprüche umfasst.
6. Teig nach Anspruch 5, welcher ausserdem einen Fettstoff, vorzugsweise 100 g Fett oder weniger per kg Mehl und  
10 noch bevorzugter 15 bis 30 g Fett per kg Mehl enthält.
7. Teig nach einem der Ansprüche 5 und 6, welcher 25 bis 1250 Pilzamyase-Einheiten (PAE) der  $\alpha$ -Amylase per kg Mehl und vorzugsweise 75 bis 250 PAE der  $\alpha$ -Amylase per kg Mehl enthält.
- 15 8. Teig nach einem der Ansprüche 5 bis 7, welcher 25 bis 500  $\beta$ -Xylanase-Einheiten der Hemicellulase per kg Mehl und vorzugsweise 35 bis 250  $\beta$ -Xylanase-Einheiten der Hemicellulase per kg Mehl enthält.
9. Teig nach einem der vorhergehenden Ansprüche, welcher 450 bis 4000 Lipase-Einheiten per kg Mehl und vorzugsweise 500 bis 2500 Lipase-Einheiten per kg Mehl enthält.
- 20 10. Teig nach einem der vorhergehenden Ansprüche, welcher im wesentlichen von Emulgatoren frei ist.
11. Verfahren zur Brotherstellung, welches das Herstellen eines Teigs nach einem der Ansprüche 5 bis 10 und das Backen des Teigs umfasst.

25

Revendications

1. Composition d'amélioration du pain, qui comprend au moins une lipase, au moins une hémicellulase et au moins  
une amylase.
- 30 2. Composition suivant la revendication 1, dans laquelle la lipase provient d'une souche de Rhizopus, Aspergillus, Candida, Penicillium ou Mucor.
- 35 3. Composition suivant la revendication 1 ou 2, dans laquelle l'hémicellulase provient d'une souche d'Aspergillus ou de Trichoderma.
4. Composition suivant l'une quelconque des revendications précédentes, dans laquelle l'amylase est une  $\alpha$ -amylase et provient, de préférence, de Aspergillus oryzae.
- 40 5. Pâte qui comprend une composition suivant l'une quelconque des revendications précédentes, de la farine, de l'eau et de la levure.
6. Pâte suivant la revendication 5, qui comprend, en outre, une matière grasse, de préférence, 100 g ou moins de matière grasse par kg de farine et, avec avantage, de 15 à 30 g de matière grasse par kg de farine.
- 45 7. Pâte suivant l'une quelconque des revendications 5 ou 6, qui comprend de 25 à 1250 unités amylase fongique (UAF) d' $\alpha$ -amylase par kg de farine et de préférence, qui comprend de 75 à 250 UAF d' $\alpha$ -amylase par kg de farine.
8. Pâte suivant l'une quelconque des revendications 5 à 7, qui comprend de 25 à 500 unités  $\beta$ -xylanase d'hémicellulase  
50 par kg de farine et de préférence, qui comprend de 35 à 250 unités  $\beta$ -xylanase d'hémicellulase par kg de farine.
9. Pâte suivant l'une quelconque des revendications précédentes, qui comprend de 450 à 4000 unités lipase par kg de farine et de préférence, qui comprend de 500 à 2500 unités lipase par kg de farine.
- 55 10. Pâte suivant l'une quelconque des revendications précédentes, qui est sensiblement exempte d'agents émulsifiants.
11. Procédé de préparation de pain, qui comprend la formation d'une pâte suivant l'une quelconque des revendications 5 à 10 et la cuisson de la pâte.